ZOOLOGICAL SCIENCE 10: 863–867 (1993)

[RAPID COMMUNICATION]

Isolation of Rat GnRH Receptor cDNA having Different 5′-Noncoding Sequence

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ABSTRACT—A rat GnRH receptor cDNA was cloned with 1317 base pairs (bp) from a pituitary cDNA library. This clone had a full amino acid coding region, with 172 and 161 bp noncoding sequences in 5′- and 3′-flanking regions of the coding region, respectively. The nucleotide sequence of amino acid coding region was completely identical to that of previous report by Kaiser et al. (1992), but 5′-noncoding region was much longer than the previously reported one and its sequence was different. This 5′-noncoding region could be divided into two subregions on the basis of similarity between the two sequences. The 32 bp sequence flanking the coding region was completely identical to the previous report, but remaining 5′-end sequence almost totally lacked similarity. In the 3′-flanking region of coding region, 167 bp-long sequence was also newly identified. The new species of GnRH receptor mRNA found in the present study suggests that the presence of various mechanisms of GnRH receptor expression in the rat pituitary.

INTRODUCTION

Gonadotropin-releasing hormone (GnRH) stimulates gonadotropin release and biosynthesis in pituitary gonadotropes [1, 5, 6]. Its receptor has been characterized pharmacologically [8] and by ligand binding studies [15]. On the other hand, GnRH receptors have been reported in various organs, including the hippocampus [3], ovary [12, 13], testis [7], and thymus [14]. However, as yet little information is available whether there are many kinds of molecular species of GnRH receptor or not.

Recently, GnRH receptor cDNA was cloned from αT3, gonadotropic cell line derived from mouse pituitary, and the cDNA was functionally expressed in *Xenopus* oocytes [16, 18]. The nucleotide sequence of GnRH receptor revealed that the receptor belongs to G-protein-coupled receptor family that has seven transmembrane domains. And recently human [11] and rat [10] GnRH receptors were also isolated by conventional DNA hybridization using probes generated by PCR, which were designed on the basis of mouse GnRH receptor. We have also isolated rat GnRH receptor, but it has a different sequence from that reported by Kaiser *et al.* [10] in 5′-noncoding region.

MATERIALS AND METHODS

Preparation of a probe for screening by polymerase chain reaction (PCR)

A pair of oligonucleotide primers were designed to cover full amino acid coding region based on the sequence of mouse GnRH receptor cDNA [16, 18]. Sequences of the primers were sense 5′-GGGAATTCTGTCCTTGGAGAAATA TG-GCTAACAAT-3′ and antisense 5′-GGGTACCT-TATTTCTATCTGAGTTCT TGTGTAGTC-3′. Each oligonucleotide has restriction site in the 5′-end (*Eco*RI or *Kpn*I, respectively). Total RNA was prepared from adult mouse pituitaries by the method of Chomczynski and Sacchi [4], and cDNA

Accepted August 4, 1993
Received June 30, 1993
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was prepared with Superscript reverse transcriptase (GIBCO BRL, USA) for PCR template. PCR amplification of cDNA by using the primers described above and AmpliTaq (Perkin Elmer Cetus, USA) was performed for 30 cycles (94°C for 1 min; 60°C for 1 min; 72°C for 1 min), and yielded 1 kilobases (kb) products. The PCR products were cloned into *Eco*RI and *Kpn*I sites of pUC118/119 and sequenced to check validity of the reaction. This PCR-generated mouse GnRH receptor cDNA was ^{32}P-labelled by random hexamer priming (Ready-to-Go DNA labelling kit, Pharmacia, Sweden) and used as a probe for library screening.

Screening library of rat pituitary cDNA

The adult male Sprague-Dawley rat pituitary cDNA library in the cloning vector λgt11 was purchased from Clontech (USA). Independent 10^6 plaques of the library were screened by the conventional filter hybridization methods [17] with the mouse GnRH receptor probe described above. After three rounds of screening of the library, one positive plaque was isolated and purified. *Eco*RI and *Sac*I sites were used for subcloning into pUC 118/119. One of *Eco*RI sites (3′ end of the insert of the λgt11 cloning site) of the clone was destroyed. Overlapping restriction fragments were subcloned (Fig. 1) and sequenced by dideoxy-chain termination method using single-stranded DNA (produced by the helper phage, M13KO7) and Sequenase ver. 2.0 kit (United States Biochem., USA). The reason of destroyed restriction site was a deletion of 34 base pairs from λgt11 adjacent to the cloning site.

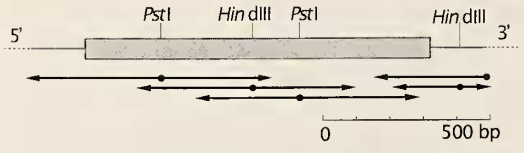

Fig. 1. DNA sequencing strategy for the rat pituitary GnRH receptor cDNA. The shadowed box indicates the predicted coding region of GnRH receptor. Restriction sites for *Pst*I and *Hind*III, which used to sequencing, are indicated.

RESULTS AND DISCUSSION

A new rat GnRH receptor cDNA has been cloned. The size of the clone was 1.3 kb (Fig. 1), and its nucleotide sequence and deduced amino acid sequence are shown in Figure 2. This clone has entire amino acid coding region with extended sequences to 5′- and 3′- termini; 172 bp of 5′- and 161 bp of 3′-flanking regions, respectively. Recently a rat GnRH receptor cDNA sequence has been reported, which contained full coding sequences and 73 bp-long 5′-noncoding region [10]. Compared with this report, 5′-flanking region of coding region found in the present study was longer and different. However, adjacent 32 bp sequence was completely identical to the previous report, and remaining 5′-end sequence totally lacked similarity. On the other hand, 3′-noncoding sequence was additional one which was newly found. For this cloning, we used Sprague-Dawley rat pituitary cDNA library which was also used by Kaiser *et al.* [10]. The present result together with the findings of Kaiser *et al.* [10] suggests that there are at least two cDNA populations with the same amino acid coding sequence in the rat pituitary gland.

Figure 3 shows a comparison of 5′- and 3′-noncoding sequences in some mammalian species (rat, mouse and human). In 5′-noncoding nucleotide sequence, there are two subregions with differences in identity. High similarity region exists between -36 and -1; it is marked (a) in Figure 3A, and the identity of the rat sequence is 92% to mice and 72% to human sequence. However, remaining 5′-end region lacks similarity. On the other hand, 3′-noncoding sequence shows 73 and 58% identity to mice and human sequences, respectively (Fig. 3B).

There was no possible translational initiation site in 5′-noncoding region of the open reading frame. One ATG site (-173) was on the open reading frame, but termination codons at -114 and -84 were present before the suggested initiating site. The mouse cDNA reported by Tsutsumi *et al.* [18] also contained a termination codon before the first ATG on the open reading frame. Our new GnRH receptor cDNA shows that there are at least two GnRH receptor mRNAs in the rat

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          TT  GCCGGTTTCC  AGCCGCAGTT  TAATAAATGG  GTAGAGTTAC  TGACCGATCC -121
 TGGTGTTAAC  GGGATGGCAC  GCGACGTGGT  GCTCTCTGAT  GCGATGATGG  GCTATCTCCA  -61
 TTTCATTGCA  AATATTCCGG  TCAAAGGCAC  TCGACTCTTG  AAGCCCGTCC  TTGGAGAAAT   -1
ATG GCT AAC AAT GCG TCT CTT GAG CAG GAC CAA AAT CAC TGC TCA GCC ATC AAC AAC AGC   60
 M  A  N  N  A  S  L  E  Q  D  Q  N  H  C  S  A  I  N  N  S
ATC CCC CTG ACA CAG GGC AAG CTC CCG ACT CTA ACC TTA TCT GGA AAG ATC CGA GTG ACG  120
 I  P  L  T  Q  G  K  L  P  T  L  T  L  S  G  K  I  R  V  T
GTG ACT TTC TTC CTT TTC CTA CTC TCT ACT GCC TTC AAT GCC TCT TTC TTG GTA AAG CTG  180
 V  T  F  F  L  F  L  L  S  T  A  F  N  A  S  F  L  V  K  L
CAG AGG TGG ACC CAG AAG AGG AAG AAA GGA AAA AAG CTC TCA AGG ATG AAG GTG CTT TTA  240
 Q  R  W  T  Q  K  R  K  K  G  K  K  L  S  R  M  K  V  L  L
AAG CAT TTG ACC TTA GCC AAC CTC CTT GAG ACT CTA ATC GTC ATG CCG CTG GAT GGG ATG  300
 K  H  L  T  L  A  N  L  L  E  T  L  I  V  M  P  L  D  G  M
TGG AAC ATC ACT GTT CAG TGG TAT GCT GGA GAG TTC CTT TGC AAA GTT CTC AGC TAT CTG  360
 W  N  I  T  V  Q  W  Y  A  G  E  F  L  C  K  V  L  S  Y  L
AAG CTC TTC TCT ATG TAT GCC CCA GCC TTC ATG ATG GTG GTG ATT AGC CTG GAT CGC TCC  420
 K  L  F  S  M  Y  A  P  A  F  M  M  V  V  I  S  L  D  R  S
CTG GCC GTC ACT CAG CCC TTA GCT GTC CAA AGC AAG AGC AAG CTT GAA CGG TCT ATG ACC  480
 L  A  V  T  Q  P  L  A  V  Q  S  K  S  K  L  E  R  S  M  T
AGC CTG GCC TGG ATT CTC AGC ATT GTC TTT GCG GGA CCA CAG TTA TAT ATC TTC AGG ATG  540
 S  L  A  W  I  L  S  I  V  F  A  G  P  Q  L  Y  I  F  R  M
ATC TAC CTA GCC GAC GGC TCT GGG CCA GCA GTT TTC TCG CAA TGT GTG ACC CAC TGC AGC  600
 I  Y  L  A  D  G  S  G  P  A  V  F  S  Q  C  V  T  H  C  S
TTT CCG CAA TGG TGG CAT GAA GCC TTC TAC AAC TTT TTC ACC TTC AGC TGC CTG TTC ATC  660
 F  P  Q  W  W  H  E  A  F  Y  N  F  F  T  F  S  C  L  F  I
ATC CCT CTT CTC ATC ATG CTA ATC TGC AAT GCC AAA ATC ATC TTC GCC CTC ACA CGA GTC  720
 I  P  L  L  I  M  L  I  C  N  A  K  I  I  F  A  L  T  R  V
CTT CAT CAG GAC CCA CGC AAA CTA CAG CTG AAT CAA TCC AAG AAT AAT ATC CCA AGA GCA  780
 L  H  Q  D  P  R  K  L  Q  L  N  Q  S  K  N  N  I  P  R  A
CGG CTG AGA ACT CTA AAG ATG ACA GTG GCA TTT GCC ACC TCC TTT GTC ATC TGC TGG ACT  840
 R  L  R  T  L  K  M  T  V  A  F  A  T  S  F  V  I  C  W  T
CCC TAC TAC GTC CTA GGA ATC TGG TAC TGG TTT GAT CCG GAA ATG TTA AAC AGG GTG TCA  900
 P  Y  Y  V  L  G  I  W  Y  W  F  D  P  E  M  L  N  R  V  S
GAG CCA GTC AAT CAC TTC TTC TTT CTC TTT GCT TTT CTA AAC CCG TGC TTC GAC CCA CTT  960
 E  P  V  N  H  F  F  F  L  F  A  F  L  N  P  C  F  D  P  L
ATA TAT GGG TAT TTC TCT TTG TAA TTGGGA  GACTACCCAA  GCACTTGTGC  TGAAACCCAT 1020
 I  Y  G  Y  F  S  L  stop
 ATACGGGATA  ACTTATGTCA  CCGGTTGAGA  ATAAGCTCAA  AGCTTGCAAC  ATACCTATGT 1080
 GCAAGCAAGG  CAGGGTTTGG  GCTCAGGTTA  GCAGCCTTGT  CCATATACAG  AGTTTGTTGT 1140
 TAGCG                                                              1145
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FIG. 2. Full sequence of the rat pituitary GnRH receptor cDNA and deduced amino acid sequence of the protein. Nucleotides are numbered, starting with the initiating codon, ATG. Negative numbers represent 5′-noncoding region.

pituitary gland with different nucleotide sequences in 5′-upstream noncoding region with identical amino acid coding region. Northern hybridization experiment also showed heterogeneity in the size of GnRH receptor mRNA of rats [10] and mice [16].

GnRH receptor is widely distributed in tissues and concerned with various physiological activities [5]. Interested in the effect of GnRH on the cytodifferentiation of gonadotropes, we applied GnRH on rat pituitary primordia in organ culture, and found that LH-immunoreactive cells were responsive at 13.5 days of gestation (unpublished observation). In fact, it was reported that GnRH receptor was present in the pituitary *in situ* on 13.5 days of fetal life [2, 9]. Whether the receptor was the products of heterogeneous mRNA or not was left unanswered. To solve this question, the knowledge on genomic DNA is needed.

ACKNOWLEDGMENTS

This study was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture, Japan to S. K. and M. K. P.

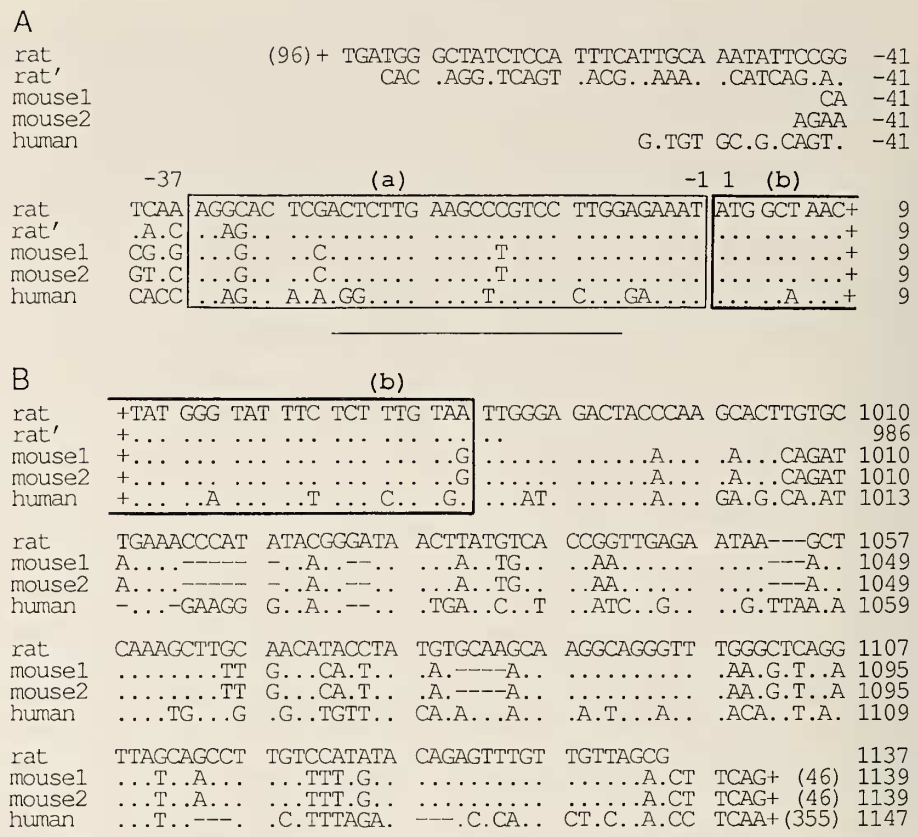

FIG. 3. Comparison of nucleotide sequences of 5′ (A)- and 3′ (B)-noncoding region of rat [10], mouse [16, 18] and human [11] GnRH receptors. The sequence of rat GnRH receptor reported by Kaiser *et al.* [10] is shown in the second line with "rat'" title. The sequences titled "mouse1" and "mouse2" are reported by Tsutsumi *et al.* [18] and Reinhart *et al.* [16], respectively. Dots represent identical bases with our rat GnRH receptor. Dashes represent deletion of bases. Box (a) is conserved region among these species. Box (b) is predicted amino acid coding region.

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